IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF: **XUEYING HUANG, *ET AL.***	
APPLICATION NO.: **10/630,248**	GROUP ART UNIT: **1762**
FILED: **JULY 30, 2003**	EXAMINER: **JIMMY LIN**
FOR: **MICROPARTICLE-BASED METHODS AND SYSTEMS AND APPLICATIONS THEREOF**	ATTORNEY DOCKET NO.: **CL 1943 US NA**

DECLARATION UNDER 37 C.F.R. § 1.131

COMMISSIONER FOR PATENTS
P.O. BOX 1450
ALEXANDRIA, VA 22313-1450

Sir:

1. I Ming Zheng, am a co-inventor with Dr. Xueying Huang[1] in the above-identified patent application.

2. I obtained a Bachelor's degree in Electronics from Peking University, People's Republic of China in 1984 and a Ph.D. in Chemistry from Princeton University in 1995. I was a post-doctoral fellow at the National Institute of Health from 1996 to 2000.

3. I am currently employed by the Central Research and Development Department (hereinafter "CR & D") of E. I. du Pont de Nemours & Co., Wilmington, DE, United States of America (hereinafter "DuPont"), as a Research Associate. I joined DuPont in 2000. DuPont is the assignee of the above-referenced patent application.

[1] *See* accompanying declaration from Dr. Xueying Huang, also under 37 C.F.R. § 1.131.

4. While working at C R & D of DuPont, in relation to the above-referenced patent application and otherwise, I used gold nanoparticles coated with ethylene glycol oligomer, and gold nanoparticles coated with a mixture of ethylene glycol and other ligands, that were made by Dr. Xueying Huang, to examine protein binding specificities. Said gold nanoparticles were prepared by Dr. Xueying Huang in 2001-2002, also working at DuPont CR &D, in Wilmington, DE, USA, at that time. Dr. Xueying Huang is no longer employed by DuPont.

5. In the Non-Final Office Action mailed on July 03, 2006, and subsequently in the Non-Final Office Action mailed on June 07, 2007 the Examiner rejected Claims 2, 5-17, and 19 under 35 U.S.C. § 103(a) as being obvious over Templeton, *et al.*, Langmuir 15:66-76 (1999), in view of Foos, *et al.*, Chem. Mater. 14:2401-08 (2002). Specifically, the Examiner asserted that "it would have been obvious to one of ordinary skill in the art at the time of the invention to have used an ethylene glycol oligomer in the preparation of water-soluble gold nanoparticles of Templeton because Foos teaches that an ethylene glycol oligomer can increase the water solubility of a gold nanoparticle."

6. I, Ming Zheng, declare that in September of 2001, at CR & D of DuPont in the United States, Dr. Xueying Huang and I reduced to practice the following entity:

water-soluble, metallic nanoparticles having a mixed monolayer of
(i) a capture-coating component and
(ii) a shielding component;

which was prior to the online publication date of Foos, *et al.* (April 19, 2002). Further to this declaration, I attach notebook pages signed by Dr. Xueying Huang and witnessed by co-workers as Exhibits 3H-8H, wherein the dates have been redacted. Also attached are pages from my notebooks (Exhibits 1 & 2), which have been signed by me and witnessed by my co-workers at DuPont.

Exhibit 1 exemplifies the reduction to practice of water-soluble, metallic nanoparticles made through Ligand Exchange reactions. For example, the data in the lower half of the page show two gel shift assays indicating the absence of non-specific protein binding on to ethylene glycol (abbreviated as "EG-SH" in the notebook page) coated Au particles. A protein called GST (noted by the gene name "pET41a" in the notebook page) was used for the assay. This protein has a GSH binding domain. When mixed with GSH coated Au particles ("Au-GSH"), we observed band shift as shown by lanes "1" in both gel images. When the GSH was exchanged by EG-SH, such band shift disappeared, as shown by lanes "2" in the "4% TBE" gel image, and lanes "2" and "3" in the "1% TBE" gel image. These data indicate that EG acts as an efficient shielding component.

Similarly, Exhibit 2 exemplifies the reduction to practice of water-soluble, metallic nanoparticles made through the ligand exchange reactions. In the previously submitted declaration under 37 C.F.R. § 131, dated December 03, 2006, I, Ming Zheng had erroneously stated that Exhibit 2 exemplifies nanoparticles made through **direct synthesis** reaction. By way of the present declaration, I, Ming Zheng hereby correct said erroneous statement. The correct statement is that Exhibit 2 exemplifies the reduction to practice of water-soluble, metallic nanoparticles made through the **ligand exchange** reactions. Particularly, the data in the upper left gel image show that a mixed monolayer with both EG and GSH at the ratio of 1: 6 provides specific binding (band shift with GST protein), yet resists non-specific binding (no band shift with BSA and streptavidin).

Although Exhibits 1 and 2 do not explicitly demonstrate that the final concentration of water in the reaction mixture for the direct synthesis of Au particles with ethylene glycol coating is from about 9% to about 18% V/V, as required by independent Claim 2, as suggested in the "Conclusions" of Exhibit 7H, it is clear that Dr. Xueying Huang and I were cognizant of the importance of the concentration of water to the stability of gold nanoparticles. Particularly, the "Conclusions" (See bottom of notebook Page Exhibit 7H) in Exhibit 7H, which is

dated February 25, 2002, i.e., prior to the effective date of the Foos reference, states that:

1. **Without CH_3COOH, control of $NaBH_4$ could lead to Au~~~EG_4 (a few drops of $NaBH_4$ solution) nanoparticles (purple). It is not stable in H_2O. After days (5~10), some ppt was formed.**

2. **With CH_3COOH, control of $NaBH_4$ is still needed. pH: 2.0→ 5.0? More $NaBH_4$ could be tolerated in the formation of Au~~~EG nanoparticles. Stability?**

The absence of CH_3COOH in the first conclusion (i.e., higher concentration of water) and presence of CH_3COOH in the second conclusion (i.e., lower concentration of water) and Xueying Huang's comments about stability indicate his cognizance of the importance of the concentration of water to the stability and yield of the gold nanoparticles with ethylene glycol coating.

The concentration range of 9-18% V/V of water is only a preferred range of water concentration for direct synthesis. Secondly, the approach in Foos relates to ligand exchange. Foos does not relate to the direct synthesis Method. Moreover, Foos does not disclose or discuss the water concentration range or its importance in stability of gold nanoparticles. In fact, Foos relates to ligand exchange reactions, and the water content and its implications are relevant only in direct synthesis method.

The direct synthesis of gold particles coated with ethylene glycol, and ethylene glycol mixed with other ligands was developed and optimized over some period of time, starting no later than November 7, 2001 (see Exhibit 3H, line in the middle of the page), and with first sign of success around February 25, 2002 (See Exhibit 4H, Exhibit 5H TEM image of the $Au(EG)_4$ particle, and Exhibit 6H-8H on exploring conditions for stable particle formation).

7. I, Ming Zheng, also declare that although Exhibits 1, 2, 3H-8H, demonstrate the reduction to practice of the present invention for representative coated metallic nanoparticles, I believe that Dr. Xueying Huang and I have demonstrated

reduction to practice for the claimed coated metallic nanoparticles because (i) the binding specificity and (ii) the resistance to non-specific binding are rendered by the choice of ligand and the function of ethylene glycol oligomers. The chemical identity of the core metal does not play a role here because the metal core is buried or shielded by the coating and does not interact directly with the environment.

As a person signing below:

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true. I also declare that all statements were made with knowledge that willful false statements, and the like, are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and any such willful false statements may jeopardize the validity of either the patent application or any patent issuing thereon.

Respectfully Submitted,

9/7/07

Ming Zheng Date

THE FOLLOWING PAGE IS

EXHIBIT 1

TITLE _____ DATE _____

PURPOSE _____ E100993-105

Protein titration experiment with protein staining

Sample 1. 3μl Au (7/26/01 prep. 15:1)

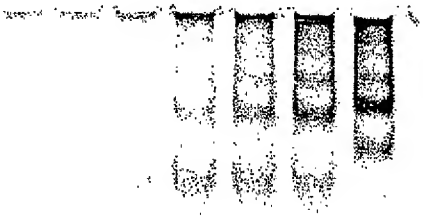
2. 3μl Au + 2μl PED 107-6 7/27/01's prep fraction 2, column 2

3. " + 4

4. " + 6

5. " + 8

6. " + 10 μl PET41a



6% polyacrylamide gel, 1hr, 90v

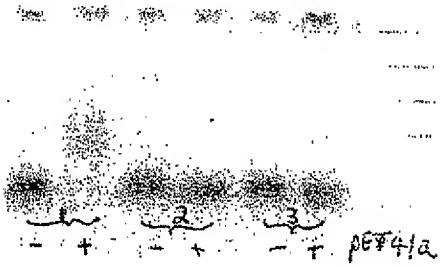
12well gel

Testing Xueying's ligand exchange samples.

Xueying made Au-GSH particles last Thursday

Today he did the following test

1. Au-GSH + THF
2. Au-GSH + EG-SH in H_2O
3. " " " in THF



1% TBE, 90v, 40 min

4% TBE, 90v, 40 min

EXPERIMENTER _____ DATE _____

WITNESSED BY _____ DATE _____

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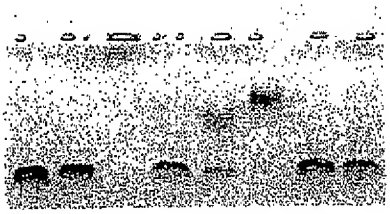
EXHIBIT 2

1% TBE, 90 v , 32 min

1. 2 ul of 11/14/01, Au-EG/GSH 1:5
2. 1+ 6 ul PET41a (GST)
3. 1+ 6 ul PED107-6 (GST-ZFP)
4. 2 ul of 11/14/01, Au-EG
5. 1+ 6 ul PET41a (GST)
6. 1+ 6 ul PED107-6 (GST-ZFP)

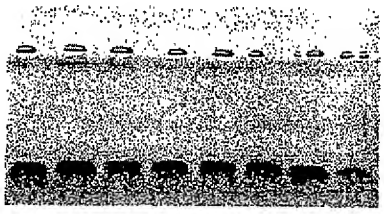
[protein] ~ 1 mg/ml



1% TBE, 90 v , 32 min

1. 1 ul of 9/11/01, Au-EG/GSH 1/6, 40-50% fraction
2. 1+ 6 ul BSA
3. 1+ 6 ul Lysozyme
4. 1+ 6ul Streptavadin
5. 1+ 6 ul PET41a (GST)
6. 1+ 6 ul PED107-6 (GST-ZFP)
7. 1+ 6 ul PED107-6 (10/22/01's , ~0.2 mg/ml)
8. 1+ 6 ul PED107-6 (10/22/01's , dialyzed, <0.2 mg/ml)

All other proteins ~ 1 mg/ml



1% TBE, 90 v , 32 min

1. 2 ul Au-EG/GSH 5:1
2. 1+ 6 ul PET41a (GST)
3. 2 ul of 11/14/01, Au-EG/GSH 1:1
4. 3+ 6 ul PET41a (GST)
5. 2 ul of 11/14/01, Au-EG/GSH 1:5
6. 5+ 6 ul PET41a (GST)
7. 1 ul of 9/11/01, Au-EG/GSH 1/6
8. 8+ 6 ul PET41a (GST)

pI for a few proteins:

Zif268	pI=9.9	10.9 charge at pH7	90 a.a
NRE	pI=9.8	10.9 charge at pH7	93 aa.
pET41a	pI=6.4	-4.0 charge at pH7	285 a.a.

EXPERIMENTER Ming DATE

WITNESSED BY Kevin Arnold Smith DATE

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EXHIBIT 3H

TITLE _____ DATE _____

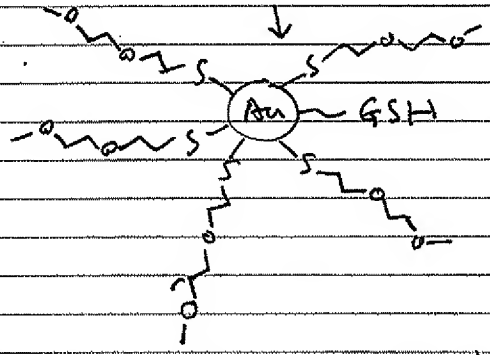
E 101228- 10 PURPOSE _____

Suggestions and Ideas

① Minimize charge effect

Synthesis of a neutral surface

$HAuCl_4$ + HS~O~O⁻ + GSH



Broad size distribution needs to be narrowed.

Method : non-polar solvent

H_2O / MeOH

H_2O / EtOH

H_2O / Acetone

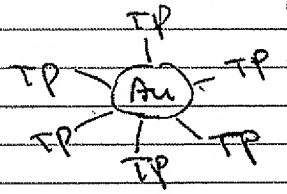
H_2O / iPrOH

⋮

ⓐ Direct synthesis ? Tried before, not working.

ⓑ Replacement reaction ?

How to quantify the number of GSH ?



+ HS~O~O⁻ + GSH → Further separation leads to

Ratio: 50 : 1

25 : 1

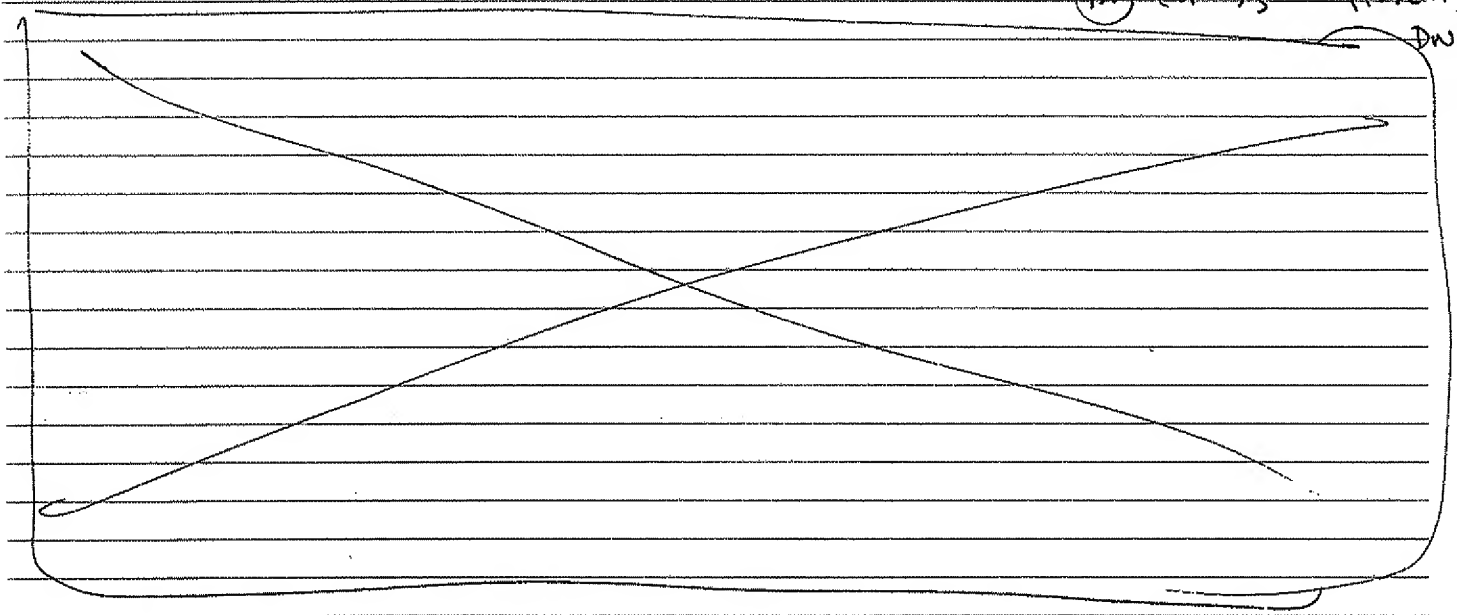
15 : 1

$Au-(TP)_n-GSH$

$Au-(TP)_n-(GSH)_2$

$Au-(TP)_n-(GSH)_3$

GSH could be any other ligands, such as protein, DNA, et al.



EXPERIMENTER Guangjin Zhang DATE _____

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EXHIBIT 4H

TITLE Bioelectronics DATE

PURPOSE Direct synthesis of (Au)~EG4 **E101228- 57**

Reaction:

$HAuCl_4$ + HS~ $\xrightarrow[NaBH_4 / H_2O]{MeOH / Acetic\ acid}$ (Au)~EG4

Recipe:

		work or not
MeOH	12 mL	
CH_3COOH	2.0 mL	
$HAuCl_4$	0.0759 (0.2 mmol)	X
EG4-SH	7.47 mg ($\frac{0.2}{6}$ mmol)	
$NaBH_4$	0.2 g	
H_2O	10.0 g	

- procedures are the same as tioproinin
- Result:
precipitate was formed when $NaBH_4$ solution was added.
This recipe seems not work!

Recipe:

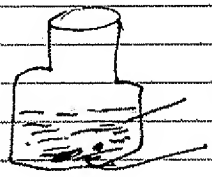
H_2O	14 mL
$HAuCl_4$	0.0759 (0.2 mmol)
EG4-SH	7.47 mg ($\frac{0.2}{6}$ mmol)
$NaBH_4$	0.2 g
H_2O	10.0 g

clear
divided the solution into sever portions.

① when a little volume of NaBH solution (several drops) wa added, no precipitate was formed. ⇒ water soluble materi

② When more $NaBH_4$ solution was added, precipitate was formed. ⇒ Black ppt. does not dissolve in H_2O

Sit on the hood for days.



yellow (light color)
deep yellow precipitate

(a) Ran gel. (Au)~EG4 moved

(b) TEM

EXPERIMENTER DATE

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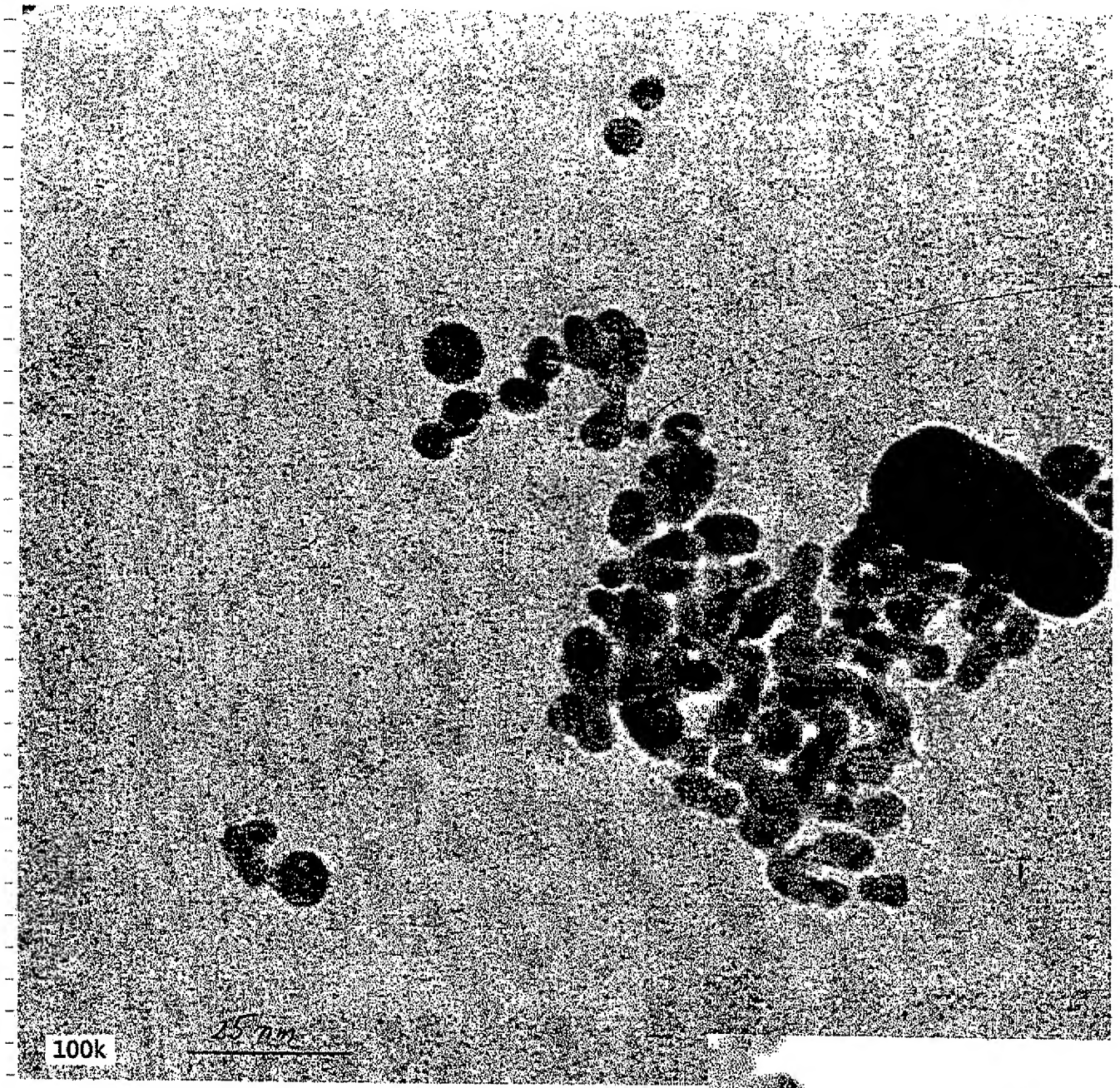
EXHIBIT 5H

TITLE Bioelectronics DATE

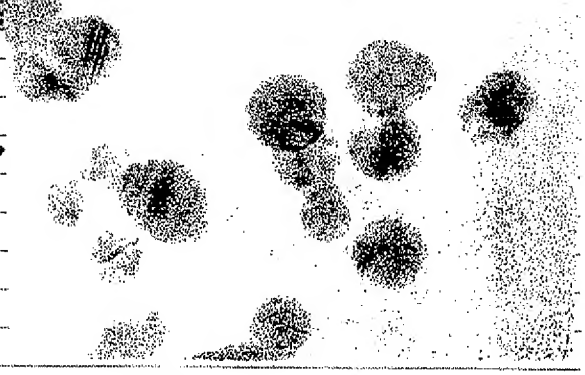
E101228- 58 PURPOSE TEM of (Au)~EGa

Direct synthesis of (Au)~EGa

TEM by Rick Li at CCAS



different area →



EXPERIMENTER Yueqing Wang DATE

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EXHIBIT 6H

TITLE Bioelectronics DATE

PURPOSE Direct Synthesis of (Au)~EG4

E 101228- 59

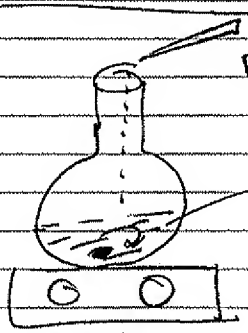
Recipe:

H2O (nanopure)	14 mL
CH3COOH	0.5 mL
HAuCl4	0.075 g (0.2 mmol)
EG4-SH (10mg/mL)	28.4mg (0.1 mmol)
NaBH4	0.1 g
H2O	5.0 mL

Dropwise added NaBH4 solution.

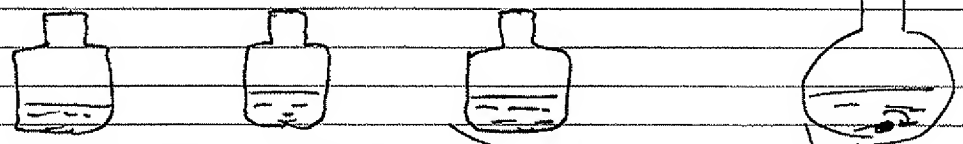
1/3 of original material

many bubbles were formed.



Fractions

#1 #2 #3 #4



After a few drops at the beginning, took ~0.5mL solution. The solution was clear, purple. However, it gradually changed into grey and ppt was formed.

After 6-7 drops, took ~0.5mL solution. It was clear and purple. Still stable after 24h.

After 1.0mL NaBH4 added, took ~0.5mL solution. Clear & purple. After 1 day, material sink to the bottom of sample vial.

Added ~2.0mL NaBH4. Color is grey. After 1 day, the par is easy to sink down stirring for 24h.

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EXHIBIT 7H

TITLE Bioelectronics DATE

E101228-60 PURPOSE Direct synthesis of (Au)~EG4

Recipe:

$HAuCl_4$ —(H_2O / CH_3COOH / EG4-SH)→ (Au)~EG4

—($NaBH_4$ / H_2O)→

H_2O	5.0 g*
✓ CH_3COOH	0.1 mL
$HAuCl_4$	0.025 g
EG4-SH	5.0 mg
$NaBH_4$	0.035 g
H_2O	2.0 g

← added 5.0 g H_2O

$\Delta V \approx 10$ mL

After ~1.0 mL $NaBH_4$ was added, the solution became grey. A lot of bubbles were formed.

H_2O	5.0 g
CH_3COOH	0.5 mL
$HAuCl_4$	0.025 g
~~EG4-SH~~	~~5.0 mg~~
$NaBH_4$	0.035 g
H_2O	2.0 g

pH ≅ 2.0

No EG4-SH

When $NaBH_4$ was added, ppt was formed immediately.

Did not work

Conclusions:

① Without CH_3COOH, control of $NaBH_4$ could lead to (Au)~EG4 (a few drops of $NaBH_4$ solution) nanoparticles (purple). It is not stable in H_2O. After days (5~10), some ppt was formed.

② With CH_3COOH, control of $NaBH_4$ is still needed. pH: 2.0 → 5.0 ? More $NaBH_4$ could be tolerated in the formation of (Au)~EG4 nanoparticles. Stability ?

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EXHIBIT 8H

PURPOSE

Run gel by Dr. Ming Zheng:

control
NaBH4
amount!

1 2 3 4 5

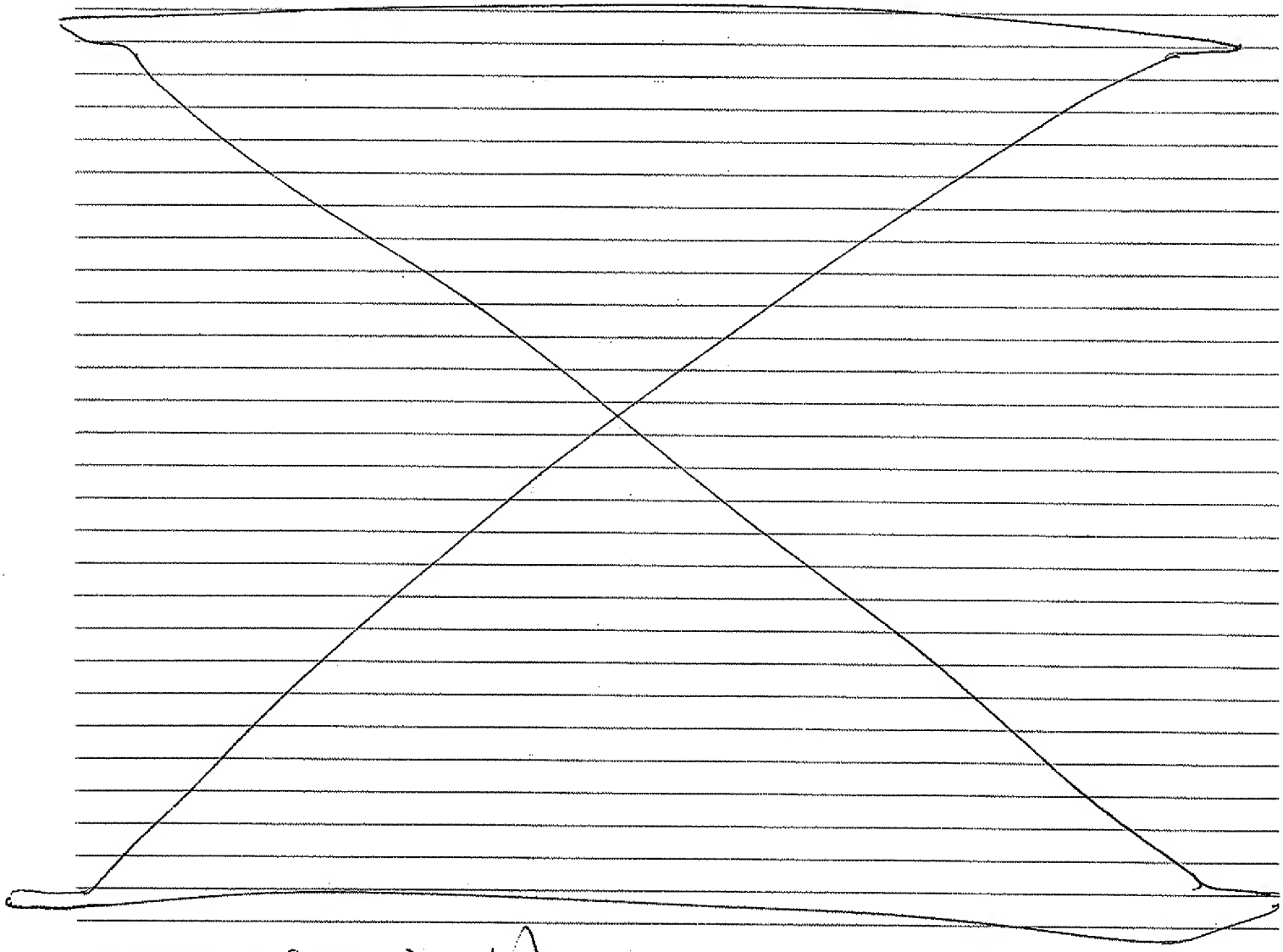
4% TBE, 90v, 40 min

1. Au-EG4, #2
2. Au-EG4: #3
3. Au-EG4: #4
4. Pt-Tp
5. Ag-Tp

① channel 4: (Pt)~TP can not be injected.
Reason: (Pt)~TP is in MeOH.

② (Ag)~EG4 #2 seems working this agrees with Rick Li's TEM image.

③ (Ag)~TP seems also working. Different color?



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